

Reports

Simple Laboratory Method for Producing Enriched Carbon-13

With the increased precision in the measurement of relative isotope abundance ratios afforded by improvements in isotope-ratio mass spectrometers (1, 2), it is possible to carry out a number of different tracer experiments using isotopes at much lower enrichment levels than were formerly required.

For the case of C^{13} enriched by a factor of 1.5 over the 1.1 percent natural abundance level (thus, 0.55 atom percent excess C^{13}), assuming a mass spectrometric sensitivity of 0.01 percent (1) in the C^{13}/C^{12} ratio, dilution by a factor of 5500 is allowable. A dilution factor of 500 would probably be the practical limit because of complications from the possible isotope effects (3) in the chemical manipulations.

A simple, inexpensive laboratory method for producing enriched C^{13} (as barium carbonate) is reported here (4). The general technique (5) makes use of the difference in rates of isotopic reactions. When a competitive irreversible isotopic reaction is carried out to a high extent of completion, the residual unconverted substrate becomes exponentially enriched in the heavier isotope.

If one defines the isotope rate factor $\alpha = k/k^*$ as the ratio of the rate constants for the reactions of the light and heavy isotopic reactants, then for the case of "small isotope effects" ($\alpha - 1 \ll 1$), the over-all separation factor is given (5, 6) by $S = \alpha^N$, where N is the equivalent number of stages of separation, defined $N = -\ln(1 - \theta)$; θ is the fraction of sub-

strate reacted, or the "cut"; and the separation factor S is the isotopic ratio (heavy/light) in the enriched residual substrate divided by the corresponding ratio in the initial substrate. Thus, for 99 percent reaction, the cut $\theta = 0.99$, and $N = 4.61$; for $\theta = 0.999$, $N = 6.91$, so that a considerable amplification of the single-stage isotope effect is possible using high cuts.

The reaction of dehydration of formic acid with sulfuric acid was found to be a suitable one for C^{13} enrichment by the high-cut method. Ropp, Weinberger, and Neville (7) reported a C^{14} isotope effect at 24.75°C of $k_{14}/k_{12} = 0.9141$, from which one may estimate a value of $k_{12}/k_{13} = 1.047$. Some very recent experiments by Yankwich and Haschemeyer (8) on the C^{13} isotope effect lead to an extrapolated value at 300°K of $k_{12}/k_{13} = 1.0576$. As the basis of a sample calculation, the value $\alpha = 1.057$ will be used. To produce 1 mole of 1.4-percent C^{13} ($S = 1.273$) requires a value of $N = 4.35$, corresponding to $\theta = 0.9872$; thus about 80 moles of formic acid are needed. For 0.1 mole of 1.6-percent C^{13} , $N = 6.75$, so that about 85 moles would be required.

In this study, eight batch-type experiments were carried out, with a number of minor variations in procedure. The initial quantity of formic acid ranged from 1.2 to 132 moles. All the experiments were performed at room temperature (about 25°C), after an initial period at 0°C . The procedure for the largest-scale run (132 moles) was as follows.

Fifteen pounds of formic acid (General Chemical Co., 88 to 90 percent) were cooled to 0°C ; 18 lb of precooled sulfuric acid (technical, 93 percent) were added slowly to the cold formic acid in a large open vessel. The operation was carried out under a hood. After 5 hr at 0°C , the rate of evolution of carbon monoxide had greatly diminished; the solution was allowed to warm up to room temperature and was transferred to a 12-lit flask. During the next 48-hour period, approximately 55 lb of sulfuric acid was added in several increments. After a total of 92 hours,

the rate of gas evolution was quite slow (about 3 to 5 ml/min); 5 lb of sulfuric acid was added and the first product collection was begun. The carbon monoxide was swept out of the flask by bubbling a slow stream of dry, CO_2 -free air through the solution. The gases then passed through an Ascarite tube (to remove possible CO_2 formed from some trace impurity), then through a copper oxide tube (450°C) into a gas-washing bottle containing 1/1 ammonium hydroxide. After 13 hours, the collection was ended and the C^{13} -enriched carbonate was precipitated (barium chloride solution), washed, filtered, and dried. One-tenth mole of barium carbonate was recovered. The CO_2 evolved from a sample of this BaCO_3 was 33.2 percent enriched (9) in relation to a reference sample of CO_2 obtained from quantitative oxidation of formic acid (which itself happened to be 1.1 percent enriched in relation to tank CO_2). From this it can be calculated that the residual formic acid should be characterized by a separation factor $1.057 \times 1.332 = 1.408$ (40.8 percent enriched) at the mean time of the CO_2 collection (about 98 hours after the start of the reaction). Two subsequent samples of enriched BaCO_3 (14 and 1.2 g) were collected over a period of 1 week. A final sample (0.4 g) was obtained by heating the flask to 65°C for 1 day to decompose the residual formic acid. The C^{13} enrichments were 40.8, 51.9, and 52.6 percent, respectively, so that the weighted average enrichment of the last three samples was 42.0 percent. Calculating back to the average enrichment at the mean time of the first CO_2 collection, a result of 38.6 percent is obtained; this is in fair agreement with the estimated value based on the enrichment of the first CO_2 sample collected.

Detailed results of all the experiments and a quantitative comparison with theory are presented elsewhere (6). It will suffice to say that, although the yields of enriched C^{13} were always somewhat lower than those predicted (the yields corresponded to an apparent value of $\alpha = 1.050$ rather than 1.057, probably because of handling losses), it was possible to prepare molar quantities of barium carbonate at an enrichment of 25 percent, decimolar amounts enriched to 45 percent, and centimolar quantities enriched by a factor of 1.5 above the natural abundance level.

Assuming current prices for reagent-grade formic acid and technical-grade sulfuric acid, the reagent cost for a 100-mole experiment is about \$8; the use of technical-grade formic acid would reduce this cost to about \$3.30. Since no elaborate equipment is needed, and since the process requires virtually no attention, it may be of some practical

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interest as a simple laboratory technique for the production of tracer C^{13} . The possible application of the high-cut method to the enrichment of other isotopes is being considered.

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Lung Volume of Amphibian Tadpoles

In 1949 Witschi (1) described, in tadpoles of the genus *Rana*, a pair of fibrous strands which connect the round windows of the otic capsules with the bronchi. In the following year (2) he noted that larvae of *Xenopus* possess small diverticula of the bronchi which also come into contact with the round windows of the ears. Witschi called these structures bronchial columellae and bronchial diverticula, respectively.

It occurred to Witschi (1, 3) that these connections of the gas-filled lungs with the ears might serve for the transmission of pressure changes, particularly sound vibrations. In order to make quan-

titative statements about sound and pressure reception by the lung, the lung volume is an important quantity to be known.

Since dissection of the lungs and subsequent determination of their volume introduces uncontrollable errors because of manipulation, it was decided to devise a technique for measurement in the living animal.

Let a specimen of *Rana* species be swimming free in a vessel under atmospheric pressure P_1 . The volume of the animal's lung may be denoted by V_1 . Then, if the vessel is partially evacuated to pressure P_2 , the volume of the lung (assuming passive behavior of this organ) will increase to V_2 , such that

$$V_1 P_1 = V_2 P_2$$

(temperature T constant). By suitable manipulation, this expression may be restated

$$V_1 = \left(\frac{P_1}{P_1 - P_2} - 1 \right) (V_2 - V_1)$$

Since P_1 and P_2 are known, it is necessary to know only $(V_2 - V_1)$, or the increase in volume. To measure this quantity, one or more animals were enclosed in a small vessel completely filled with water. Through the stopper of this vessel, a calibrated capillary protruded, which was approximately half filled with water. Thus, the only free air present in the system is the air in the lungs of the animals. This small vessel was put in a larger vessel, in which a volume of water served as a temperature buffer, so that no changes in temperature would occur during an experiment; a thermometer was added to check this. The large vessel was then evacuated to a preset pressure in the range of 56 to 76 cm-Hg, and the rise in level of the fluid in the capillary was measured. Since the capillary was calibrated, the rise in level could be read directly as 10^{-2} ml increase in volume.

It was assumed that the lung expanded passively; if this assumption is correct, the same value for V_1 must obtain from any set of P_1 and P_2 , or in a graphical representation, the relation $(P_1 - P_2)$ versus $(V_2 - V_1)$ should be a straight line. For each animal or set of animals, such a straight line was obtained. Figure 1 shows four cases, two of *Rana catesbeiana* and two of *Xenopus laevis*. It is apparent that the slope of a particular relation is determined by the initial volume, V_1 , which is indicated in the graph. In a series of measurements on single *Rana catesbeiana* larvae of stages 28 to 29 (Witschi, 4) values of V_1 ranged from 0.14 to 0.42 cm^3 , with the mean at 0.28 cm^3 . *Xenopus laevis* larvae of similar age were measured in groups of three, four, and ten individuals, and the total lung volume was divided by the number of animals. Thus, values of V_1

ranged from 0.020 to 0.034 cm^3 with the mean at 0.026 cm^3 . Since *Rana* larvae are heavier than water and tend to sink to the bottom, while *Xenopus* larvae are lighter and have to make continuous effort to keep from floating up (5), one is led to infer that *Xenopus* larvae carry relatively more air than *Rana* tadpoles. It is then meaningful to express the lung volume as percentage of body volume. Therefore, the body volume of the animals was measured before each pressure experiment by dropping them in an appropriate graduated cylinder and noting the rise of the meniscus. Thus it appears that *Rana* larvae have an average lung volume amounting to 2.3 percent of body volume; *Xenopus* larvae, on the other hand, maintain an average of 3.7 percent air.

From these results, it is possible to obtain an upper and lower bound of the specific gravity of the tadpole as a whole. Assuming a floating condition (specific gravity, 1.0000), where the air just cancels the weight of the tissue in excess of the weight of an equal volume of water, one may calculate from the quoted percentages that the specific gravity lies between 1.020 and 1.034.

A measurement was carried out to determine the specific gravity of *Xenopus* larvae in the following manner. A small number of larvae were homogenized in a known volume of water. The total volume was determined after homogenization, which thus yielded the volume of tadpole tissue. From this 1- cm^3 samples were weighed; after correction for the added water, these weighings averaged at 1.025 for the specific gravity of *Xenopus* larval tissue (6).

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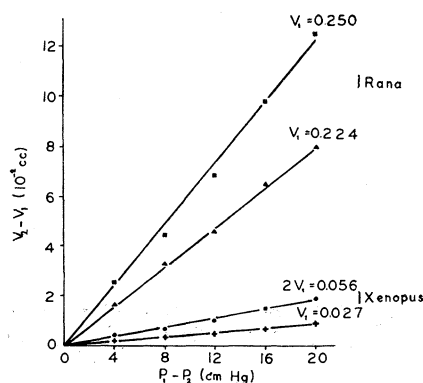


Fig. 1. Relation of applied pressure difference ($P_1 - P_2$) to lung volume increase ($V_2 - V_1$) in larvae of *Rana* and *Xenopus*. The calculated original lung volume is indicated in cubic centimeters at each graph. Note that one curve for *Xenopus* represents two animals ($2V_1 = 0.056 \text{ cm}^3$).

Leaching of Carbohydrates from Plant Foliage as Related to Light Intensity

Modern use of radioactive isotopes has effectively demonstrated the loss of mineral nutrients from plant foliage by leaching with aqueous solutions (1, 2). In addition, large amounts of organic materials, principally carbohydrates, can